CONCISE REPORT

Differential Expression of LFA-1 Molecules in Non-Hodgkin’s Lymphoma and Lymphoid Leukemia

By Giorgio Inghirami, Rosemary Wieczorek, Bang-Ying Zhu, Robert Silber, Riccardo Dalla-Favera, and Daniel M. Knowles

We investigated the expression of adherence molecules lymphocyte function–associated antigens-1α and -β (LFA-1α, -β) and p150, 95 in 103 well-characterized non-Hodgkin’s lymphomas (NHLs) and lymphoid leukemias (LLs). We found that NHLs and LLs differentially express LFA-1 molecules according to their lineage derivation, degree of clinical aggressiveness, and anatomic site of involvement. Specifically, (a) T-cell neoplasms nearly always express these molecules; (b) diffuse aggressive B-cell NHLs and mature LLs often lack LFA-1α molecules; and (c) B-cell chronic lymphocytic leukemia (CLL) is often LFA-1α-negative while B-cell small lymphocytic lymphomas (SLLs) are nearly always LFA-1α-positive. Furthermore, the low expression of LFA-1α in CLL is related to the low degree of homotypic lymphocyte adhesion after tumor promoter antigen stimulation that does not modulate the expression of LFA-1α in vitro. The differential expression of LFA-1 by B-cell CLL and SLL and their degree of homotypic lymphocyte adhesion may account for the distinct anatomic compartmentalization and characteristic clinical behavior of these two morphologically and immunologically similar lymphoid malignancies.

MATERIALS AND METHODS

Pathologic specimens. Representative samples of lymph nodes, peripheral blood, and aspirated bone marrow involved by NHL and LL were collected during the course of diagnosis in 103 patients. Each lymphoid neoplasm was classified according to conventional clinical and histopathologic criteria. Lineage assignment was determined by immunophenotypic analysis.

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**Immunophenotypic analysis.** We investigated each of these 103 pathologic specimens for their expression of the β chain and distinct LFA-1 and p150, 95 α chains by using monoclonal antibodies TS1/18, TS1/22, and Leu-M5, respectively. The expression of surface and cytoplasmic immunoglobulin, HLA-DR antigens, terminal deoxynucleotidyl transferase, and a large panel of B- and T-cell-associated antigens (CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD15, CD20, CD21, CD23, CD25, CD38, CD45) also were investigated by using previously described immunofluorescent cytofluorometric and immunoperoxidase immunohistochemical techniques. Specific fluorescence intensity was calculated as previously described.  

**Homotypic lymphocytic adhesion.** A quantity of 1 × 10⁶ mononuclear cells obtained from peripheral blood or cell suspensions prepared from the lymph nodes of patients with CLL and SLL contained ≥95% neoplastic cells and were cultured in a 24-well plate with and without TPA (50 ng/mL) for seven days. The degree of aggregation was scored as follows: no cells in clusters, 0; -10% cells in clusters, 1+; -50% cells in clusters, 2+; -100% in small clusters, 3+; and -100% of cells in large, compact clusters, 4+.

**RESULTS**

LFA-1α and/or -β molecules were present in all 29 T-cell and in 53 of 74 B-cell neoplasms (Table 1). Twenty-five of 26 small lymphocytic and follicular (low- and intermediate-grade) B-cell NHLs but only four of 13 large cell and Burkitt's (diffuse aggressive) B-cell NHLs expressed LFA-1 molecules. Sixteen of 22 LLs representative of the mature stages of B-cell differentiation and chronic lymphocytic and hairy cell leukemia (HCL) were LFA-1α–negative. Furthermore, while only five of 17 CLLs expressed LFA-1α molecules, 13 of 14 SLLs did so. In fact, the only LFA-1α-negative SLL in this series exhibited significant secondary involvement of the peripheral blood and was associated with a monoclonal gammopathy. The p150, 95 molecule detected by monoclonal antibody Leu-M5 was expressed by five of 29 T-cell and 20 of 74 B-cell neoplasms. The vast majority of the p150, 95–positive neoplasms (mycosis fungoides, CLL, SLL, HCL, and myeloma) are representative of mature stages of T- and B-cell differentiation. Eight of 17 CLLs but only five of 14 SLLs expressed p150, 95. Eight of 14 SLLs were weakly positive (23% ± 16%) for interleukin-2 (IL-2) receptors, while only two of 17 CLLs were IL-2 receptor–positive. On the other hand, CD21, CD23, CD24, and LFA-3 antigen expression was similar in both CLL and SLL. In all cases, the β₂ chain was detected in conjunction with LFA-1α and/or p150, 95. Only 38% of CLLs but 64% of SLLs expressed 100% compact aggregates (≥3+) in the homotypic adhesion assay (Table 2). Notably, only CD5-negative CLLs and SLLs exhibited spontaneous aggregation (2+). The degree of aggregation in both CLL and SLL was always related to the specific fluorescence, which is an indirect measurement of the number of membrane molecules. The relationship between LFA-1α intensity and its possible modulation by TPA in the homotypic lymphocyte adherence assay was studied in three CLLs and three SLLs that expressed variable specific fluorescence for LFA-1 (Table 3). The degree of aggregation was scored after seven days, and the cells were stained for the presence of LFA-1α or -β and p150, 95, HLA-DR, β-microglobulin (HLA-ABC), CD3, and CD5 also were analyzed at time 0 and after seven days. No significant increase in specific fluorescence was found for LFA-1α (Table 3), CD5, HLA-DR, or HLA-ABC (data not shown). An increase in p150, 95 was noted. The degree of aggregation of these six cases was also a function of each specific fluorescence intensity.

**DISCUSSION**

The results demonstrate that (a) aggressive NHLs and LLs variably express integrin receptor molecules; (b) these molecules are more often retained by T-cell than by B-cell malignancies; (c) diffuse aggressive B-cell NHLs and mature LLs tend to express LFA-1 molecules weakly or not.

**Table 1.** CD11a, c and CD18 Expression in NHLs and LLs

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Cases</th>
<th>LFA-1α (CD11a)</th>
<th>LFA-1β (CD18)</th>
<th>p150, 95 (CD11c)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B-cell neoplasms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>CLL</td>
<td>17</td>
<td>5</td>
<td>9</td>
<td>8</td>
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<tr>
<td>HCL</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>SLL</td>
<td>14</td>
<td>13</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>1</td>
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<tr>
<td>Large cell lymphoma</td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Burkitt's lymphoma</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>45</td>
<td>53</td>
<td>20</td>
</tr>
<tr>
<td><strong>T-cell neoplasms</strong></td>
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<tr>
<td>Acute lymphoblastic leukemia</td>
<td>2</td>
<td>2</td>
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</tr>
<tr>
<td>Mycosis fungoides</td>
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<td>13</td>
<td>5</td>
</tr>
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<td>Peripheral T-cell lymphoma</td>
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</tr>
<tr>
<td>Large cell lymphoma</td>
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<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>74</td>
<td>82</td>
<td>25</td>
</tr>
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</table>
LFA-1 IN LYMPHOID NEOPLASMS

all; and (d) B-cell CLLs usually express no or only low numbers of LFA-1α molecules while their tissue counterpart, the morphologically and immunologically similar B-cell SLLs, nearly always express high numbers of LFA-1α molecules. Moreover, the specific fluorescence of LFA-1α molecules, which is related to the number of membrane molecules,19 is correlated with the degree of homotypic lymphocyte adherence after TPA induction in both CLL and SLL. However, TPA does not modulate the expression of LFA-1 in vitro as previously suggested.19

LFA-1, LFA-2, and LFA-3 molecules are involved in cytolytic T-cell–target cell conjunction. Recent studies have shown that LFA-1 molecules on target as well as on effector cells play a role in cytolytic T-cell–mediated killing.20 Specifically, the lack of LFA-1 molecules from Burkitt’s lymphoma cells appears to be crucial in their escape from the host’s immunosurveillance mechanism.20 In support of this hypothesis, we found that LFA-1 molecules are expressed much less frequently by diffuse, aggressive than by low- or intermediate-grade B-NHLs. However, both indolent and aggressive T-cell neoplasms express lymphocyte integrin receptors, which suggests that the expression of these adherence molecules may differ according to lineage as well as clinical aggressiveness.

The lack of LFA-1 on aggressive lymphomas and leukemias may be explained in two ways. First, the LFA-1–negative malignant cells may represent the neoplastic counterpart of normal cell precursors that normally lack LFA-1. Alternatively, the LFA-1–negative malignant cells may have lost LFA-1 through adaptive mechanisms that provide a biologic advantage over those cells that retain LFA-1 and thereby escape from host immunosurveillance. In our studies, 13 of 15 acute lymphoblastic leukemias, representative of some of the earliest stages of B- and T-cell differentiation, were LFA-1–positive. Furthermore, B-cell CLL and SLL express virtually identical mature differentiation markers but differ strikingly in LFA-1 expression. These results suggest that LFA-1 expression is not merely linked to stages of B- or T-cell differentiation and that the lack of LFA-1 molecules by aggressive B-NHLs may be due to in vivo selection mechanisms.

Our finding that LFA-1 expression differs in CLL and SLL is particularly intriguing. Both neoplasms exhibit indolent clinical behavior, share similar and often identical morphologic and immunologic features, and are usually only distinguishable according to their anatomic sites of involvement: lymph nodes and spleen in SLL and peripheral blood in CLL. Therefore, it is possible that the presence of LFA-1 on SLL cells accounts for their preferential localization in lymph nodes and spleen and the absence of LFA-1 molecules from CLL cells and the resultant loss of cell-to-cell adherence contacts accounts for their propensity to invade the peripheral blood. The fact that the one SLL with secondary peripheral blood involvement expressed low or absent LFA-1 molecules and low to absent homotypic adhesion of CLL after TPA induction further supports this hypothesis. The delicate balance existing between the attachment and detachment of cells from the extracellular matrix and from each other determines whether a cell remains stationary, circulates, or migrates through tissue. Perhaps the lack of LFA-1 is sufficient to break this delicate balance and create the biologic difference between CLL and SLL.

The β2 and the p150, 95 α chains have recently been sequenced21,22 and cDNA probes are now available. These probes should allow us to investigate the genetic control of these molecules and further understand their role in determining the biologic differences that account for some of the clinical features observed in these lymphoproliferative disorders. However, we can speculate that the differential expression of LFA-1 in CLL and SLL may be due to selective control of the LFA-1α gene. In fact, the differences that we observed here between LFA-1 and p150, 95 expression in CLL, in vivo and after TPA induction, may indicate that the β gene is normally expressed and upregulated in conjunction with the p150, 95 gene.

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